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Chapter

Bioinorganic Chemistry and Computational Study of Herbal Medicine to Treatment of Tuberculosis

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Abstract

Tuberculosis (TB) is one of the leading infectious diseases in the world. The disease is commonly caused by *Mycobacterium tuberculosis* (Mtb) bacteria which are capable of rapidly spreading through droplet transmission. In developing countries, poverty and malnutrition cause immunodeficiency which is considered as the main risk factor for the incidence of TB. Treatment of TB has been proven to be difficult because treatment options are very limited and found to be expensive specifically in developing countries. Moreover, the existence of extensively drug-resistant TB phenomena is frequently happening in these countries because of mishandling treatments used for this disease. In Indonesia, the traditional herbal medicine, namely, jamu, has been utilized since a long time ago to treat diseases including TB. The present study by using computational methods found that there are many active compounds that can bound and influence proteins responsible for TB pathogenesis. Besides, these compounds have the potency to modulate the host immune system. The current chapter discussed the possible interaction of the antioxidant compounds with the chelating potential to form a complex with transitional metal as the central atom. In the perspective of bioinorganic chemistry, this complex has a scavenging activity which is expected to have a role in overcoming energy management of the host cell during infection pathogenesis. It is important to involve bioinorganic chemistry in energy management during infection, correlated with impairing of niacin metabolism of the host cell in which the host cell mitochondria cannot competitively gain free radicals during infection. This phenomenon is the main reason to propose herbal medicine as a source of niacin and provide a proper environment for gastrointestinal commensal microbiota to treat and govern protection from TB infection.

Keywords: bioinorganic chemistry, gastrointestinal microbiota, herbal medicine, LMWA, TB

1. Introduction

Tuberculosis is a major cause of morbidity and mortality, especially in low-income and middle-income countries [1]. The World Health Organization (WHO) mentioned in the Global Tuberculosis Report 2016 that there were approximately 10.4 million people infected with TB. Among those, 1.4 million patients lost their life leaving poor families in helpless situations. Although the mortality rate of TB patients had been decreased by 22% between 2000 and 2015, resistant and persistent types of disease remain as a major problem till present [2].

Over the past few years, TB treatment depended on the use of antibacterial compounds such as isoniazid, rifampicin, pyrazinamide, and ethambutol as first-line drugs. But recently it had proven that the anti-TB drug(s) had caused mutations in *Mycobacterium tuberculosis* (Mtb) bacteria giving rise to a new kind of multidrug-resistant TB (MDR-TB) [3]. Therefore, the treatment of the disease becomes extremely hard and forces the physicians to start using the second-line of drugs such as aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and para-aminosalicylic. These drugs were also causing mutations in Mtb which gives rise to new cases such as extensively drug-resistant TB (XDR-TB), in which Mtb become resistant to both the first- and the second-line of treatments [4, 5].

TB treatment based on eradicating bacteria. In this way, TB therapy needs to be carried out comprehensively through improving nutritional balance and immune system modulation [6]. The mechanism of pathogenesis of Mtb contains competition between host and bacteria, for iron (Fe) involves the secretion of superoxide dismutase (SOD) enzymes which is related with the bioinorganic chemistry of complex compounds that synergistically work as energy delivering system underlies [7]. The SOD enzyme indirectly becomes part of the energy and electron transfer system because of its catalytic activity which converts superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen (O_2) to minimize the toxic effects of O_2^- [8]. This is what causes Mtb to avoid the reactive oxygen species (ROS) produced by host macrophages as an immune system defense effort. But because Mtb secrete SOD, Mtb can develop well in macrophages, and to synthesize SOD by TB bacteria, it needs Fe, and the main source will be the infected host [9]. On the other hand, the host also needs Fe for hemoglobin of red blood cells [10]. This competition will significantly disturb energy transfer system of the host and will cause lack of hemoglobin, i.e., anemia [11]. As a result of that, in Mtb infection, there is an energy competition, meaning that even though Mtb and hosts do not intersect in their energy management system, the electron flow moves toward Mtb cells rather than hosts. This is due to lower electrical potential state of Mtb. When the immune system is in proper condition, Mtb get more energy to divide and migrate. The balance between cellular and metal ion components is indirect but mediated by a special set of proteins, namely, SOD, which are controlled and organized interactions of energy transfer within the mitochondria [12].

The active compound in herbal medicine that functions as a radical scavenger in bioinorganic chemistry is known as low molecular weight antioxidant (LMWA) complex. This LMWA contains metal ions such as Fe, Mn, Cu, and Zn [13, 14]. The complex can overcome the impairing energy transfer system of the host because they can transfer electron when electron transfer system is impaired due to anemia in the case of TB infection. The potential of LMWA complex as a free radical scavenger is also important as an anti-inflammation agent.

2. Computational studies of active compound of Indonesian herbal medicine used in treating TB

Computational drug discovery has been widely described as a tool and database for saving, managing, analyzing, and modeling compounds. This is proven as an effective strategy to guide and improve the discovery and development of new therapy. The real aim of drug discovery is to find new "lead" compounds and new chemical entities that could potentially be developed into new medicines, gaining the target specificity related to disease development or changing the target function. In the steps of drug discovery, computational drug discovery is involved in designing the main compounds with computer programs, identifying target and validity, and observing the effectiveness, the bioavailability, and the side effect possibility of lead compounds and lead candidates. Recently, a lot of researches have shown that the application of computational drug discovery can significantly help in identification and/or development of an effective treatment [15, 16].

In fact, computational drug discovery can also be utilized to approve traditional treatments that have been used for a long time to treat several diseases. In this case, computational drug discovery tools have been used for observing and analyzing the active compounds of Indonesian medicinal plants used for treating TB. Some studies showed that natural compounds from indigenous Indonesian medicinal plants could inhibit the incidence of TB infection by balancing immunity system and eradicating mycobacteria [17–20].

Based on computational drug discovery tools, it has been detected that Indonesian native plants such as *Euphorbia hirta*, *Foeniculum vulgare*, *Ocimum basilicum*, *Zingiber officinalle* Rosc., *Curcuma domestica*, *Plantago major*, *Curcuma*

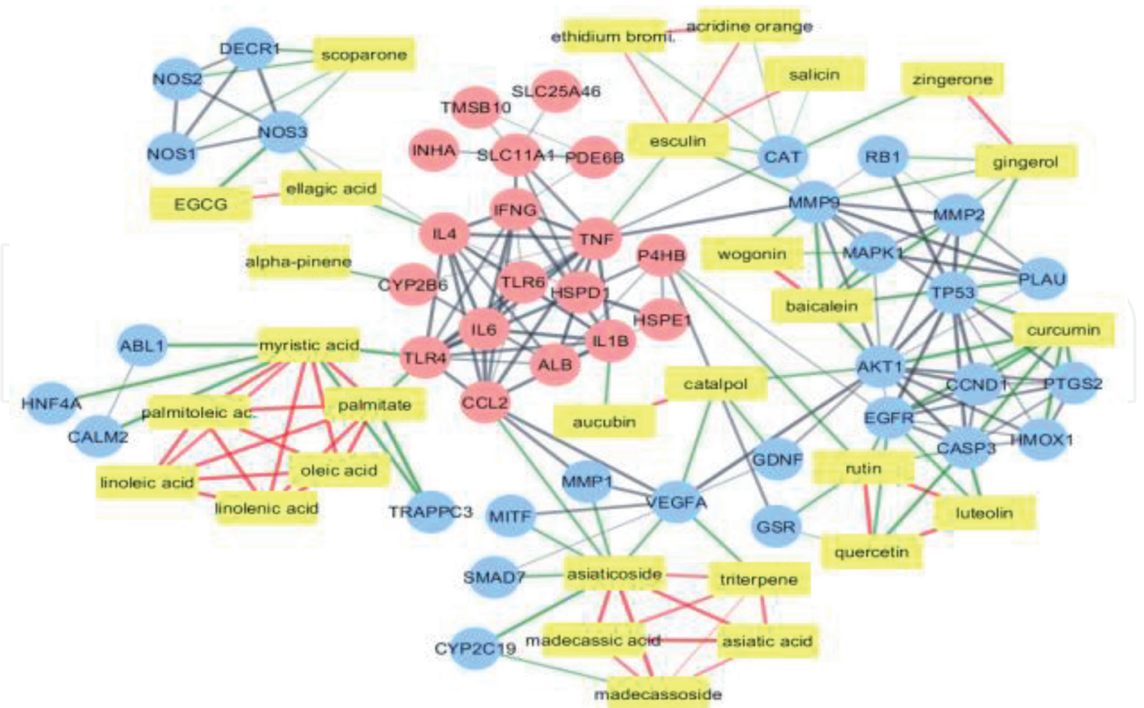

Figure 1.
Interaction between active compounds of Indonesian medicinal plants with protein-related tuberculosis. The red circle indicates protein involved in tuberculosis mechanism, while the blue one indicates protein unrelated to tuberculosis mechanism. The yellow rectangle indicates active compound. The green line indicates active compound-protein interaction. The red line indicates active compound-active compound interaction [21].

zedoaria, *Centella asiatica*, *Coffea arabica*, *Ageratum conyzoides* L., *Tamarindus indica*, *Citrus aurantifolia*, *Petiveria alliacea*, and *Lantana camara* L. contain several active compounds that interacted with protein-related tuberculosis like IL-4, TNF-α, IL-1B, CCL-2, TLR4, and P4HB (**Figure 1**); by immunity balancing they can improve the therapy outcomes and avoid Mtb reaction [21, 22]. Moreover, some studies showed that cytokines like IL-4, IL-1B, and CCL-2 could eradicate mycobacteria [23]. TNF-α, a proinflammatory cytokine, had high expression during the latent phase of infection, suggesting that it might be correlated with IFN-γ for controlling mycobacterial replication [24]. TLR4, a transmembrane protein and a member of the toll-like receptor family, has been reported to be expressed in macrophage and dendritic cell to recognize mycobacteria. P4HB, a disulfate enzyme catalyzation, increased the Th-2 cell migration. In addition, the active compounds not only interacted with protein-related immunity but also another protein like CYP2B6, a cytochrome 450 enzyme, which could be an indicator for tuberculosis therapy [25].

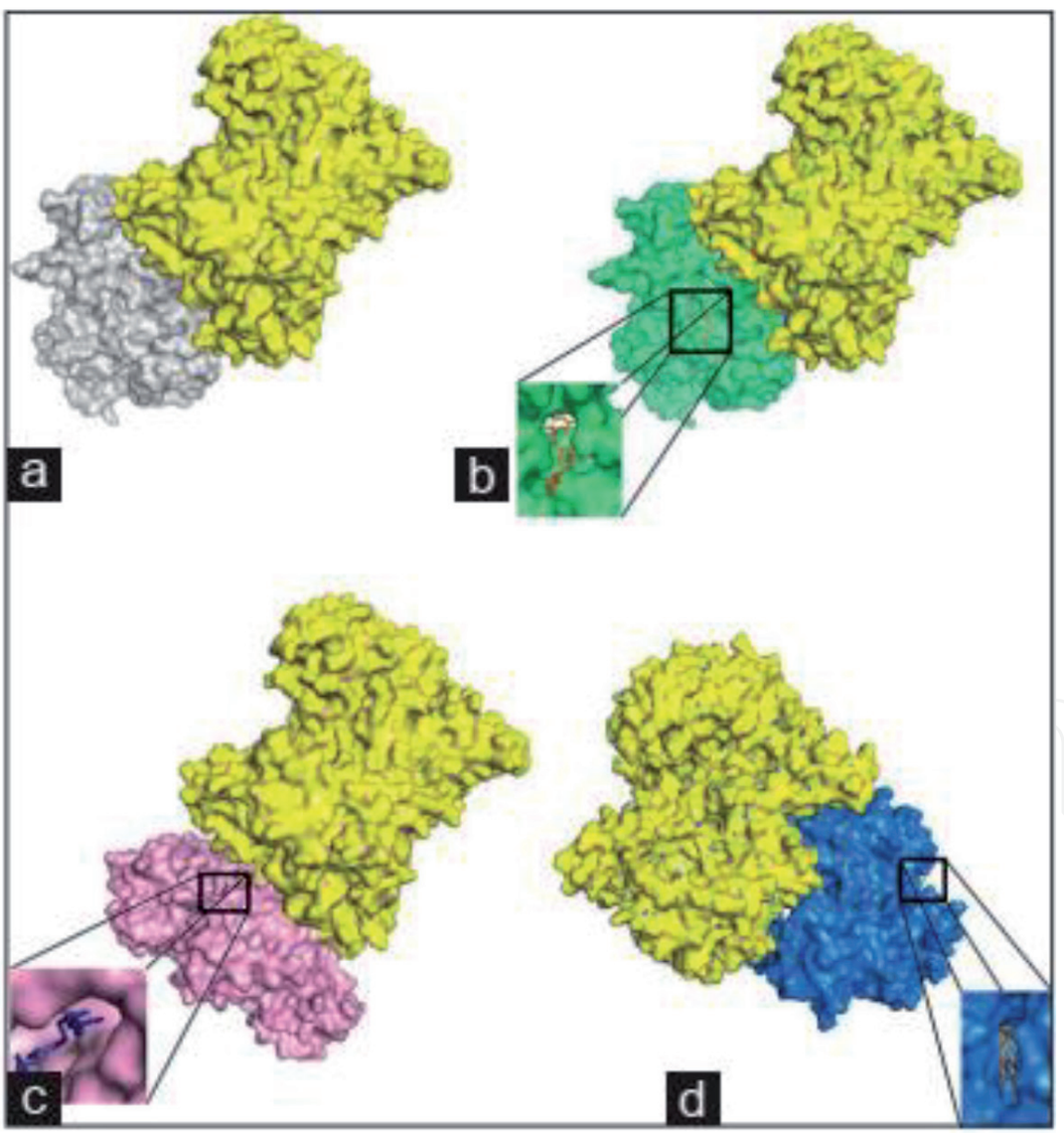

Figure 2.
Src complex. (a) Src-PI3K (wild type), (b) Src-tuberculosis drug-PI3K, (c) Src-phytol-PI3K, (d) Src-oleic acid-PI3K. Phytol and oleic acid could change the binding position of Src-PI3K, whereas the tuberculosis drug did not change the position of Src-PI3K. Gray structure, Src wild-type protein; yellow structure, PI3K; green structure, Src-drug complex; pink structure, Src-phytol; blue structure, Src-oleic complex [21].

A study conducted by Aristyani detected the incidence of interaction between active compounds extracted from Indonesian medicinal plants, *Tamarindus indica* and *Curcuma xanthorrhiza* Roxb., with Mtb's proteins by using CADD tools. Results showed that bisdemethoxycurcumin, alpha-pinene, isoamyl alcohol, furfural, lauric acid, and salicylate correlated with proteins of Mtb. Bisdemethoxycurcumin interacted with Pks 11 and Pks 18 which are involved in the α-pyrones synthesis, an essential compound in the mycobacterium cell wall. α-Pinene correlated with cyp144 which has roles for mycobacterial survival and pathogen in human cells. Both isoamyl alcohol and furfural associated with alcohol dehydrogenase proteins, while lauric acid interacted with fatty acid synthase. Salicylate bonds with various proteins such as caeA for modifying envelope structure, lpqp for encoding a membrane-bound lipoprotein, lipT for hydrolyzing from liposome suspensions,

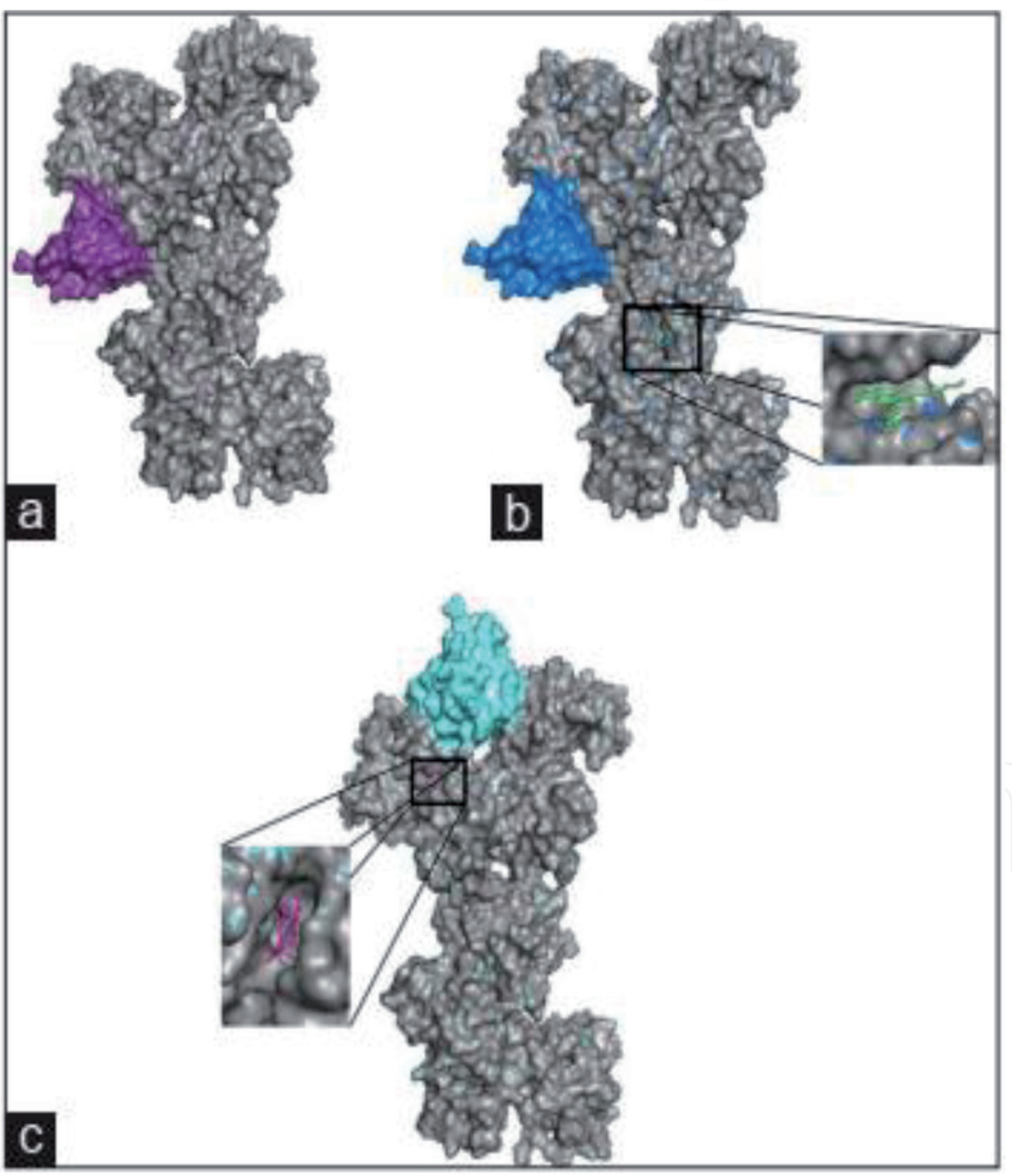

Figure 3.
PknB complex. (a) PknB-FhaA, (b) PknB-drug-FhaA, (c) PknB-phytol-FhaA. The position of FhaA was altered when PknB complexed with phytol; meanwhile, when the complex form of tuberculosis drug and PknB bound with FhaA, it had a similar position with the wild-type form. Gray structure, PknB; purple, FhaA in PknB-FhaA complex; dark blue, FhaA in PknB-drug complex; light blue, FhaA in PknB-phytol complex [21].

and estB for hydrolyzing and has peroxidase activity. Depending on that it can be concluded that natural compounds might treat tuberculosis by directly interfering the cellular system of mycobacteria [26].

A computational docking method has been also used to explore the activity of active compounds of Indonesian medicinal plants against TB. In this term, the active compounds of *Curcuma xanthorrhiza* Roxb., *Zingiber officinale* var. Rubrum, *Tamarindus indica* L., and *Citrus aurantifolia* were docked with human proteins related to tuberculosis. Results showed that tyrosine-protein kinase Src (Src), which is a non-receptor protein kinase, could reduce the survival of Mtb in macrophage; matrix metallopeptidase (MMP1), a metalloproteinase protein, can degrade the granuloma, severity of TB directly correlated with MMP1 expression [27, 28], and Mtb's proteins, protein kinase B (PknB), a transmembrane signal kinase which takes a part in mycobacteria growth and division mechanism, and KatG, a multifunctional catalase peroxynitrite; and NADH oxidase has a role in synthesizing mycolic acid of mycobacteria. The results described that active compounds such as curcumin, demethoxycurcumin, 8-gingerol, phytol, oleic acid, and linoleic acid had a higher binding scores to all the target proteins than the binding score of tuberculosis drug with the target proteins. Moreover, when the oleic acid and phytol bound with Src, it changed the binding position of PI3K, a ligand of Src. Phytol also gave a similar effect not only in Src but also in PknB. It altered the binding position of FhaA, a ligand of PknB. It might be concluded that these compounds could inhibit the activity of downstream protein target which could suppress the survival and growth of Mtb (**Figures 2** and **3**) [21].

3. Bioinorganic chemistry as a new perspective of herbal medicine to treat diseases

Recently, bioinorganic chemistry has been used in the broad areas of biological, medical, microbial, and food industry, etc. Bioinorganic chemistry is concerned about complex organic compound containing one or more metals as a center atom. These organic compounds in biological system which are bound with protein may serve multiple activities such as radical scavenging, antibacterial, antioxidant, electron transfer, or enzyme [29–33]. The section below will describe the natural bioinorganic chemistry in a plant that inspires bioinorganic compound in herbal medicine. Cause metals have a pivotal role on energy or electron transfer involves variety of small molecules, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) as electron carriers, and metalloprotein such as superoxide dismutase (SOD) enzymatic antioxidant, host-pathogen energy interaction can be explained based on bioinorganic chemistry approach.

3.1 Bioinorganic chemistry associated with radical scavenging activity

Bioinorganic chemistry study involves the role of metal complexes in biological systems, including metals which form complexes naturally with proteins (called metalloproteins) or artificially metal complexes [29, 30]. Transition metals such as iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) are required for proliferation and survival of all living organisms. They have important roles as enzymes and cofactors and environmental sensors. Iron is the most commonly used divalent metal cofactor. Iron containing enzymes or proteins is involved, among other processes, in electron transfer, maintaining redox balance, and detoxification. Hemoglobin in red blood cells is a globin protein molecule that forms a complex

with porphyrin with an iron ion center. Manganese has the strongest affinity for ATP and is the preferred cofactor in cAMP production. Zinc is used as cofactor by numerous enzymes and DNA binding proteins and additionally functions to scaffold additional proteins [34]. Enzymes such as superoxide dismutase (SOD) are enzymatic antioxidants against superoxide radicals that require Fe, Cu, or Mn for their activation. In plants, chlorophyll is a porphyrin complex with the center of magnesium ions. Secondary plant metabolites such as flavonoids possess three possible metal-chelating sites that can bind metal ions Fe, Cu, Mn, Mg, Al, Zn, and Ni [31–33].

According to bioinorganic chemistry in the case of TB pathogenesis, iron plays a pivotal role in host-pathogen interactions. Iron is one of the most important aspects needed for the initiation and establishment of infection. The mechanism of bacterial infection requires iron against host defenses, while the main component of host natural immunity limits the availability of iron for bacteria [35–38]. Mtb require iron for normal growth but faces limited metal ions because of its low solubility at biological pH and limitation of iron availability by mammalian hosts. Because of high affinity for Fe^{3+}, plasma transferrin, ferritin, and lactoferrin in extracellular fluids and polymorphonuclear leukocytes play an important role in reducing iron availability for pathogens [39]. Mtb express Fe^{3+}-specific specifications of siderophore mycobactin and carboxymycobactin to chelate insoluble metals and form host plasma proteins. Iron absorption mediated by siderophore is very important for the survival of Mtb in media with low iron levels and in macrophages. Mtb bacteria have regulation of iron absorption to maintain optimal levels of intracellular iron to prevent iron toxicity [40]. Administration of chelating agents to limit the availability of iron actually stimulates the microbes that express siderophore and carboxymycobactin to improve the acquisition of iron. Subjects who recovered from active TB tended to relapse if they received iron-rich supplements (in tonic form) compared with patients who did not receive supplements and were somewhat anemic. Limiting the availability of iron for microbes using herb medicine that form complexes with metals has a better effect to hold up bacterial proliferation [36].

For this purpose, many plant species have been used for treating tuberculosis. These herbal products have been widely proven to have an antimicrobial activity. In addition, these herbal products are used in combination with synthetic treatment to increase the efficacy of conventional drugs and also to reduce side effects and inhibit incidence of antibiotic resistance. Traditional healing systems like Ayurveda have been applied to cure TB from Africa to Asia or China and so on [41]. Almost all of these traditional herbs have been tested for their activities as antimicrobials especially on Mtb, antioxidant, as well as anti-inflammatory [41–44].

Sukmaningsih et al. [14] proved that the fruit of java plum (*Syzygium cumini*) contains naturally occurring bioinorganic compounds. The X-ray fluorescence (XRF) characterization results indicate that the primary metal contents in java plum were K, Ca, P, Cu, Ni, and Fe. The last three elements are classified as transition metals. The electron configuration of the atomic valence of Fe is $3p^6\ 4s^2\ 3d^6$, Ni is $3p^6\ 4s^2\ 3d^8$, and Cu is $3p^6\ 4s^2\ 3d^9$. The electron configuration shows that the transition metal d orbitals contain unpaired electrons that can accept electrons to occupy the orbital. The electrons interact with the molecule or specific anion through coordination bonds formed ion complex or compound. The metals Fe, Cu, and Ni that have unpaired electrons will act as the central atom and play an important role in complex formation. Analysis of LC/MS shows that the main ingredient in java plum is the flavonoid complex, namely, anthocyanins. In this compound, Fe is the center of the atom, whereas anthocyanin is a ligand. The results of the study using ESR concluded that the java plum had activity as a radical scavenger as evidenced by its ability to reduce the radical intensity of DPPH. When receiving

electrons, the anthocyanin complex does not produce new radicals as well as single compound antioxidants. This is because the metal center in the flavonoid complex has paramagnetic properties (as giving electrons) that will become diamagnetic (as get electrons) when performing its functions as free radical scavengers [14]. The mechanism activity of radical scavenging is related with delocalized electron. In the case of flavonoid and anthocyanin, the electrons came from phenolic hydroxyl group as a source of electron by denoting hydrogen atom or through the transfer of a single electron.

As reported by Aristyani, selected Indonesian medical plants used by Indonesian local people to treat TB were *Curcuma xanthorrhiza* Roxb., *Tamarindus indica* L., *Citrus aurantifolia*, and *Zingiber officinale* var. Rubrum. From the 17 selected compounds, there are 5 compounds predicted to have multitarget that are curcumin, demethoxycurcumin, phytol, oleic acid, and linoleic acid that can bind to the host metallopeptidase 1 and Src matrix proteins and PknB and catalase-peroxidase proteins from Mtb [26]. The antioxidant mechanism of these compounds is the result of interactions between phenols and transition metal ions such as copper or iron [45]. Antioxidant compound may be naturally present in different forms in the plant microstructure. Low molecular weight antioxidants (LMWA) are free from chemical or physical interaction with other plant macromolecules. LMWA are nonenzymatic antioxidant compounds capable of preventing oxidative damage by directly interacting with ROS or indirectly by chelating metals [46]. Because of its small size, LMWA pass through cellular membranes, so it can access near to molecule targets. Different nonenzymatic antioxidants can interact synergic to scavenge free radicals. Endogenous LMWA synthesized by living cells is limited, so exogenous nonenzymatic antioxidants from plant diet and phytochemicals are required. The action of LMWA in cellular metabolism can be bound as a component of endogenic enzymes, or other proteins containing transition metal could be the components of antioxidative enzymes or acting as LMWA alone and non-nutrients such as phenolic compound (phenolic acid, flavonoids, lignans), phytic acid, reduced glutathione, and melatonin to manage the free radicals by preventing free radicals to involve in Haber-Weiss reaction [46, 47].

The complex with transitional metal makes flavonoid an other organic chelating compound becoming water-soluble. This means they can interact with protein and DNA to increase bioavailability as antioxidation, antibacterial, and antitumor and affect various types of enzymatic activity [48]. Metal ions have an impact on the hydrogen atom transferring ability of the complex revealing that complexes deactivate oxidants through hydrogen atom transfer. Fe3+-primuletin (5-hydroxyflavone) is expressed as an antioxidant in in vivo system through direct scavenging of free radicals by decreasing total ROS and at the same time through enhancement of SOD and catalase activities endogenously [49].

Phenolic compounds have strong antioxidant properties in vitro, associated with their ability to trap the chain-carrying peroxyl radicals with the formation of hydroperoxides, to scavenge free radicals in Fenton reaction and chelating metal ion [50, 51]. The deprotonated phenolic group has an oxygen center that possesses a high charge density. The pKa value of most phenols is around 9.0–10.0, but in the presence of cations such as Fe(III) or Cu(II), the proton is donated at physiological pH 5.0–8.0 [52]. The ability of phenol in metal chelation can inhibit lipid peroxidation by binding metal ions to stable complex forms or preventing the interaction of metal ions with lipid hydroperoxides, which are consistently produced in living cells [53, 54]. The scavenger potencies of flavonoid metal complexes were significantly higher than those of the parent flavonoids. In addition, this metal complex also has pharmacological activities such as SOD [55].

Metal complexes have more lipophilic properties so that they easily penetrate cell membranes. The lipophilic properties of metal complexes can be explained according to coordination theory. According to the coordination molecular theory, the overlap of orbitals between metals and ligands reduces the positive charge on metal ions by accepting electrons from ligands. This causes an increase in π-electron delocalization throughout the coordination ring. This results in increased metal complex lipophilicity [56]. Therefore, research on flavonoid metal complexes is very helpful in developing new TB drugs. Accordingly, in the condition of a host of SOD depletion, flavonoid metal complex antioxidants can perform as enzymatic antioxidant SOD.

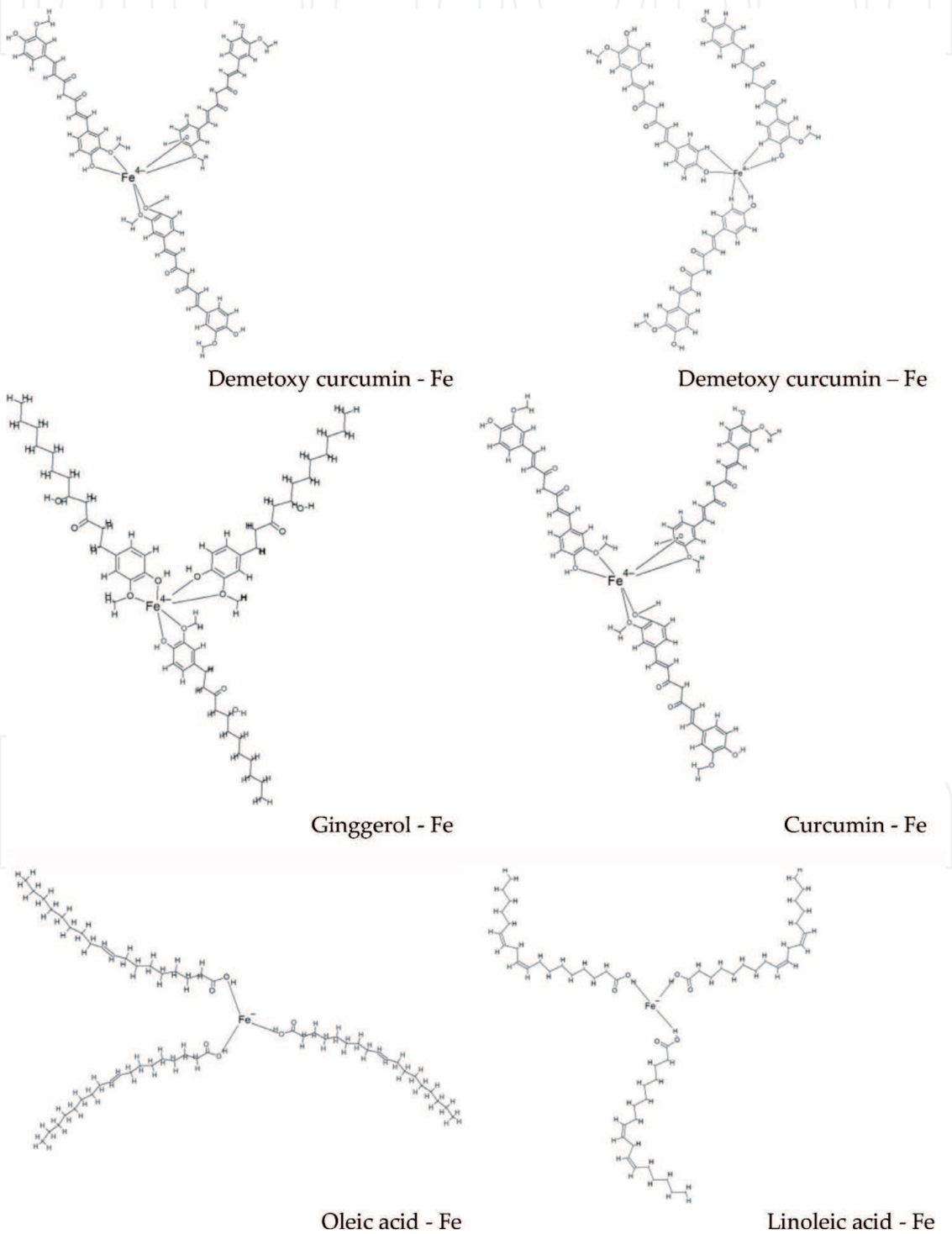

Figure 4.
Structures of the iron-demetoxy curcumin, iron -ginggerol, iron -curcumin, iron-oleic acid, and iron-linoleic acid complexes predicted by ChemSketch open babel software [57].

Given the large variety of active compounds in nature, most of them have the ability to chelate metals. In silico analysis using molecular modeling programs such as ChemSketch can be used to predict this ability. Using ChemSketch open babel software, we predicted that the chelator compounds such as curcumin, demethoxycurcumin, 8-gingerol, phytol, oleic acid, and linoleic acid can interact with transition metal Fe (**Figure 4**) [57]. It shown that the efficacy of this herbal medicine can be studied using some instrumentation such as X-ray diffraction (XRD), X-ray fluorescence (XRF), and scanning electron microscopy (SEM) and be discussed using bioinorganic chemistry perspective.

In practice, it means that herbal application for therapy can be in the form of food or drink as well as scrubbing throughout the skin. In Indonesia the herbal application for scrubbing includes fermented herbs. This scrubbing treatment aimed to leach overwhelming free radicals to sweat out the body through the skin. After several times of scrubbing, patients will gain better quality of life such as sleeping well and good appetite [58].

3.2 Bioinorganic compound associated with energy balance during TB pathogenesis

In the twenty-first century, the concept of oxidative stress has been well acknowledged in relation to understand the underlying mechanism involved in various human diseases including infectious disease such as TB [59]. Tuberculosis has been linked to free radical production either by bacteria or phagocytes. This process is closely related to the gradual loss of mitochondrial efficiency that will contribute to worsening of the disease [60].

The excessive production of reactive oxygen species (ROS) is widely associated with cellular damage. The cellular damage has been associated with depletion of coenzyme that is intricately tied to the pyridine dinucleotide. The important coenzyme in all living cells involved in oxidation–reduction reactions is known as nicotinamide adenine dinucleotide (NAD+); it is a key metabolite derived from niacin or vitamin B3 [61, 62]. NAD+ is an essential electron transporter in mitochondrial respiration and oxidative phosphorylation. NAD+ serves as an electron acceptor for glycolysis, a process that metabolizes glucose or glycogen, and is reduced to NADH. NAD+ instead of eventually transferring electrons from NADH to the mitochondrial respiratory chain. The NAD+/NADH ratio is also vital for cell physiology as it contributes to the synthesis of ATP as the energy currency of the cell. NAD+ must be regenerated from NADH for continued glycolytic flux, a process that happens within the mitochondria. If NAD+ is not regenerated, glycolysis and other metabolic pathways will stop, creating a disruption in mitochondrial metabolism and an imbalance in cellular redox homeostasis that will lead to cell death. Also NAD+ is an important precursor for the phosphorylated dinucleotide pair NADP+/NADPH in several cellular biosynthetic pathways in order to protect cells from free radicals [63]. Moreover, it plays a major role in perturbed immune responses. Thus, NAD+ dictates the host's innate and adaptive immune responses. Furthermore, it can be the strategy treatment of human diseases including TB, suggesting the potential of targeting the cellular NAD+. Four years ago, the Niederweis group described the first toxin ever found in Mtb. They found that tuberculosis necrotizing toxin (TNT) enzymatically hydrolyzes NAD+. The depletion of NAD+ inside the macrophages somehow leads to necrotic cell death of the macrophage that will cause the release of Mtb to infect more cells [64].

Mitochondrial dynamics are supposed to have an important physiological role in maintaining intracellular energy balance and energy transduction; thus, it is generally accepted that the mitochondrial electron transport chain (mETC) is the major

site for the generation of ROS. The mETC is continuously involved in reducing molecular oxygen to water in a four electron reduction processes [65]. Even a small percentage of the oxygen consumed escapes from the mETC as superoxide radical ($O2^{-}$), which can generate other endogenous ROS that will induce a great threat to aerobic organisms [66].

Herbal medicine was found to be a good source of niacin, i.e., a precursor for NAD+ synthesis. Many studies suggest that the conversion of niacin and its derivative, such as nicotinamide, was synthesized from tryptophan. The human studies have shown that 1 mg of nicotinamide is produced from 67 mg of tryptophan intake [67]. The recommended dietary tryptophan daily dose for human adults ranges from 250 to 425 mg/day, corresponding to 3.5–6 mg kg^{-1} (meanly 4 mg kg^{-1}) body weight per day [68, 69]. Thus, the herbal remedy focuses on supplementing tryptophan as precursor for niacin to enhance cofactor NAD+ to prevent the depletion of NAD+ [70]. Mammals, including humans, can synthesize vitamin B3 (nicotinamide) from tryptophan through ingestion process in the gastrointestinal system (gut and liver). Several studies report that the gastrointestinal system plays a critical role in nicotinamide supply. This was involved to the changes in tryptophan-nicotinamide metabolism, particularly on niacin nutritional status [71].

The important role of the healthy gut due to the gastrointestinal tract is prone to the attack of ROS in various diseases including infectious disease such as TB [72]. In the gastrointestinal tract, there are many major endogenous oxidative enzymatic reactions [73]. Managing oxidative stress through the healthy gut will restore the commensal microbiota for helping in tryptophan metabolism. A balanced microbial community is a key regulator of the immune response. In terms of antioxidant or free radical scavenging, tryptophan plays an important regulatory role in restoring the body antioxidant system. Tryptophan as essential amino acid must be ingested from exogenous natural sources (such as polyherbal). Increasing tryptophan concentration will increase NAD+ level in human cellular, particularly encouraging the performance of macrophage in ingestion and killing of pathogen [71, 74].

4. Role of herbal medicine in supporting gastrointestinal microenvironment during TB infection

The presence of commensal microbiota and its metabolites in the body has beneficial roles in human physiology, such as metabolism, formation of the immune system, anti-inflammatory activity, homeostasis maintenance, and vitamin production [75]. Intestinal microbiota was found to play a major role in regulating immune cell homeostasis. The explanation of "gut-lung axis" statement showed that the intestinal microbiota can regulate lung immunity and influence the lung microbiota through microbial products and immunomodulators released upon recognition of commensals and pathogens by intestinal immune cells. This explains why the reduction in commensal flora in the intestine is directly related to the severity of the inflammatory response in the lungs. This was as examined by Tsay et al. that there was a significant effect of commensal microbiota depletion on *Escherichia coli* pneumonia-induced myeloperoxidase (MPO) activity in the lungs and bacterial killing activities of alveolar macrophages by using the commensal depletion model in mutant WT and toll-like receptor 4 (TLR4) mutant mice. The study demonstrated that gut microbiota involved in stimulating lung inflammatory reaction against bacterial challenge through TLR4 which binds to lipopolysaccharide (LPS) [76, 77]. The innate immune system detects invasion of microorganisms via TLR, which can recognize microbial components and trigger an inflammatory response. TLR is a germ-coding pattern recognition receptor, and several have been

identified, such as TLR4 which recognizes LPS and TLR2 as receptor of lipoteichoic acid (LTA) [78]. In addition, the gut microbiota is important in increasing nuclear factor kappa beta (NF-κB) activation, an important early step in innate immune cell activation, in the lung through TLR4 and LPS supplements which serve to increase lung defense through the TLR4 and NF-κB signaling pathways. This confirms that gut commensal microbiota is crucial in maintaining lung defense against bacterial challenge through enhancement of alveolar macrophage activity and neutrophil infiltration [76].

Therefore, antibiotic therapy effect on intestinal microbiota may cause unbalanced inflammatory response in the lung [79]. Components of herbal medicines can improve the composition of the gut microbiota to turn in homeostasis, thus restoring dysfunction and associated pathological conditions. This is because the gut microbiota can metabolize herbal medicine chemicals and the generated metabolites have bioavailability, bioactivity, and toxicity that are different from their precursors [80]. Commonly, polar compounds are found in herbal medicine extracts, and the bioavailability of these chemicals is usually very low due to poor lipophilicity. However, the gut microbiota converts the molecules to be smaller parts that are less polar and more lipophilic [81].

Notable interactions between the active components of herbal medicines and gut microbiota are also being vigorously inspected such as glycosides affluent in many herbal medicines that always hold limited intestinal absorption because of poor intestinal permeability. The gut microbiota which are encoded with glycoside hydrolase genes can cleavage glycosyl or glucuronosyl parts from the backbone. Normally, the secondary glycosides produced by this process hold better bioavailability and thus can be absorbed properly by the intestine [82]. Biotransformation of herbal medicine components carried out by the intestinal microbiota can be done in various ways, for example, hydrolation such as deglycosylation; oxidation such as fission, hydration, hydrogenation, hydroxylation, methylation, and oxygenation; reduction such as dehydration, dehydroxylation, demethylation, decarboxylation, and dehydrogenation; rearrangement; isomerization; condensation; ester hydrolysis; esterification; and intramolecular cyclization.

Furthermore, depending on those various reaction mechanisms, the gut microbiota can trigger a series of metabolic reactions simultaneously and successively against the structural property of the herbal medicine compound. For example, glycosides in herbal medicines are normally metabolized first by gradual hydrolysis, through deglycosylation and esterlysis and then by hydrolysates generating secondary glycoside or aglycone. The secondary glycosides are further converted by skeleton-retained modification (triterpene glycosides), by skeleton fission (flavonoid glycosides), or by skeleton rearrangement (iridoid glycosides). Generally, different bacteria in the gut microbiota can cooperate to promote metabolism of a single compound, while one single bacterial strain is able to transform different compounds [83]. In the human intestinal microbial metabolism of quercitrin, an active compound of Indonesian herbal medicine for tuberculosis treatment [21] showed *Fusobacterium* K-60 deglycosylates the quercitrin, whereas four other strains of bacteria, specifically *Bifidobacterium* B-9, *Pediococcus* Q-5, *Streptococcus* S-3, and *Bacteroides* JY-6, were bounded in further fission of the quercetin (aglycone) [84]. Meanwhile, several compounds such as geniposide, aconitine, and shikonin belonging to different structural types were transformed by *Clostridium butyricum* via various reaction mechanisms: deglycosylation, dehydration, condensation, dehydrogenation, and intramolecular cyclization [82].

Interestingly, herbal medicines can modify the composition of gut microbial community while being metabolized. Some experiments have been tried using fecal transplantation to show that herbal medicine therapy targets the gut microbiota.

For example, the heat shock protein (HSP) expression levels of the murine liver and intestine were altered by a herbal medicine formula that contains *Panax ginseng* root and rhizome, *Ligusticum chuanxiong* rhizome, *Poria cocos* sclerotium, *Atractylodes macrocephala* rhizome, *Glycyrrhiza uralensis* root and rhizome, *A. sinensis* root, *Paeonia lactiflora* root, *Rehmannia glutinosa* tuberous root, *Cinnamomum cassia* cortex, and *Astragalus membranaceus* root. Thus, the alteration only occurred in intestinal microbiota rather than in germ-free mice [85].

5. Conclusion

Herbal medicine used for treating TB produces its pharmacological effect through the following. First, the immune system perspective. The target of most herbal medicine active compounds are proteins involved in the immune system. It can be indicated that these compounds have potential as an immune system modulator to treat tuberculosis diseases. Second, bioinorganic chemistry perspective. Herbal medicine has been endowed with the extraordinary ability as an antioxidant. This antioxidant is grouped as low molecular weight antioxidants (LMWA) that easily permeate to cell membranes. Bioinorganic chemistry perspective discusses LMWA-centered transition metal (such as Fe, Cu, Mn, Zn). Transition metals are directly interacting chelated by LMWA, thereby preventing them from participating in metal-based ROS production. Third, managing free radical. The collaboration between LMWA and metal ion has the ability to manage excessive electron to become moderate-level free radical that is beneficial on a host. Fourth, supporting the gastrointestinal system. The proper herbal medicine formulation will provide nutrition for NAD+ precursor on a host as well as determine gut commensal microbiota diversity that influences the microbiome in the lung. Finally, herbal medicine has a great prospect to treat TB instead of an antibiotic.

Acknowledgements

We are grateful to Dr. A.A. Alit Sukmaningsih for his valuable assistance in carrying out the prediction structure of metal complex.

Conflict of interest

The authors declare no conflict of interest.

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